IN VITRO RESPONSES OF SOFT WATER ALGAE TO FERTILIZATION

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ABSTRACT

The effects of additions of carbon, nitrogen and/or phosphorus on the growth of algae contained in a sample of surface water obtained from an oligotrophic, soft-water lake were investigated using in vitro cultures maintained in a controlled environment for at least 21 days.

Enrichment with any one substance did not stimulate algal growth. Addition of both nitrogen and phosphorus resulted in phytoplankton responses (ASU/ml) at least 100 times that of the control. No significant effect related to carbon additions was determined. In those cultures receiving both nitrogen and phosphorus but no carbon the alkalinity (mg/l $CaCO_3$) was found to have risen after three weeks from a value of 12 to 25.

A linear relationship between the organic nitrogen content of suspended solids and the standing crop of algae (ASU/ml) was obtained.

INTRODUCTION

Nutritional relationships of axenic cultures of algae in media approximating the chemistry of surface waters have been carried out since the early 1940's (Chu, 1942). More recently the concept of utilizing axenic strains of algae to assess pollution of surface waters has been advanced (Skulberg, 1964, 1967) and an extensive program, particularly in the United States, to investigate eutrophication on this basis has been initiated (Anon, 1969). Relationships between nutrient levels at the time of spring overturn and subsequent phytoplankton development in surface waters has also been suggested (Sawyer, 1947). Very little information is available however concerning concentrations in surface waters during the growing season for those nutrients considered responsible for excessive productions of algae - nitrogen, phosphorus, and more recently carbon - which will not support undesirable numbers of phytoplankton.

The following study is a preliminary investigation to assess the relationships between various levels of aqueous fertility and the responses of the phytoplankton inherent in a raw water sample when cultured in vitro in a controlled environment.

MATERIALS AND METHODS

The experimental material for this study was obtained in early November from the surface of Twelve Mile Lake, a low alkalinity body of water which does not normally support excessive quantities of phytoplankton (Christie, 1968). Characterization of the sample with respect to water chemistry and phytoplankton content were carried out according to standard procedures (Anon, 1965).

In vitro nutrient experiments were set up by adding 500 ml aliquots of thoroughly mixed raw water to acid washed sterile cotton-plugged 32 oz bottles containing various concentrations of carbon, nitrogen or phosphorus added as $NaHCO_3$, KNO_3 or KH_2PO_4. The bottles were mounted on an oscillating table located in a growth chamber maintained at $20^{o}C$ and exposed to a light intensity of 4000 footcandles in a 14:10 hour light/dark cycle.

Each culture was sampled at least weekly. The standing crop of algae, expressed as Areal Standard Units (ASU/ml), is based on enumeration of between 150-200 organisms.

Fourteen days after the beginning of the experiment 100 ml aliquots of several cultures were filtered through prewashed 0.45 micron membrane filters and the nitrogen content of the suspended solids determined.

The alkalinity of those cultures receiving no carbon additions was measured at the termination of the study.

RESULTS

Original Sample

Characteristics of the original raw water sample with respect to chemistry and phytoplankton content are listed in Table I and Table II. The N:P ration of the seston and the total phosphorus and Kjeldahl nitrogen levels have also been included.

In vitro Experiment

In the following experiment the responses of the inherent algal population to increases in the fertility of the sample of raw water by additions of carbon as $NaHCO_3$, nitrogen as KNO_3 or phosphorus as KH_2PO4 were investigated.

The growth curve of a sample which received quantities of all three nutrients is illustrated in Figure 1.

The experiment was terminated after an incubation period of 21 days and the algal standing crop associated with each treatment is listed in Table III, duplicated treatments being indicated with an asterisk. The alkalinity of those systems which received no additional carbon were also measured at that time.

The algal population and nitrogen content of the suspended solids of several samples were obtained after an incubation period of 14 days. The results of these analyses, when examined statistically were found to display a linear relationship (Figure 2).

TABLE I

CHEMICAL ANALYSIS OF RAW WATER

Ca	6
Mg	2
Na	1
K	1
Fe	0.20
SiO_2	1.14
SO_4	6
Cl	3
soluble reactive PO_4:P	0.007
total PO_4:P	0.02
NO_3:N	0.06
NO_2:N	0.005
NH_3:N	0.04
organic N	0.12
alkalinity (mg/l $CaCO_3$)	12.0
phenol	0.00
suspended solids	2
dissolved solids	48
conductivity solids	55
pH	7.8
N:P ratio seston	9.2

TABLE II

PHYTOPLANKTON COMPOSITION OF RAW WATER

	ASU/ml
CYANOPHYTA	
Chroococus	3.08
CHRYSOPHYTA	
Dinobryon	142.29
BACILARIOPHYTA	
Synedra	0.34
Tabellaria	4.66

FIGURE 1. Growth of algae in response to additions of $NaHCO_3$ (7.5 mg C/l), KNO_3 (14.84 mg N/l), KH_2PO_4 (0.65 mg P/l).

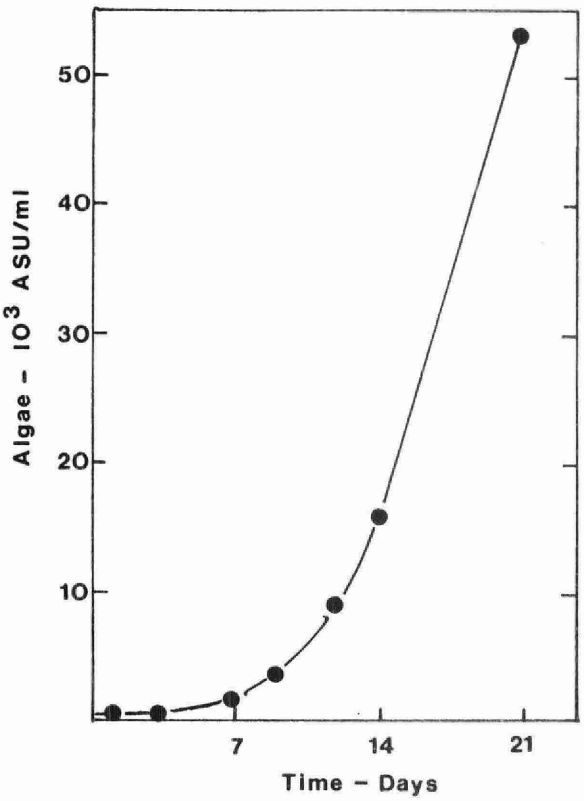

TABLE III

Phytoplankton standing crops ($\log_{10}$ ASU/ml) of cultures of lake water enriched with carbon, nitrogen and phosphorus after an incubation period of 21 days (* duplicated treatments)

Inorg. C mg/l	NO_3:N mg/l	Total Phosphorus mg/l		
		0.02	0.22	0.65
2.9	0.06	2.36		
	9.60	2.17*	4.88	
	14.84	2.17	4.28*	4.79
	20.10		4.82	4.86*
7.5	0.06	2.17		
	9.60	2.41*	4.46	
	14.84	2.24	4.65*	4.88
	20.10		4.73	4.59*
13.5	0.06	2.15	2.09	
	9.60	2.39*	4.56	
	14.84	2.56	4.64*	4.73
	20.10		4.62	4.55*

FIGURE 2. Relationship between standing crop of algae (ASU/ml) and nitrogen content of suspended solids (ug/ml).
(Y = 667.69 + 4545.58 X)

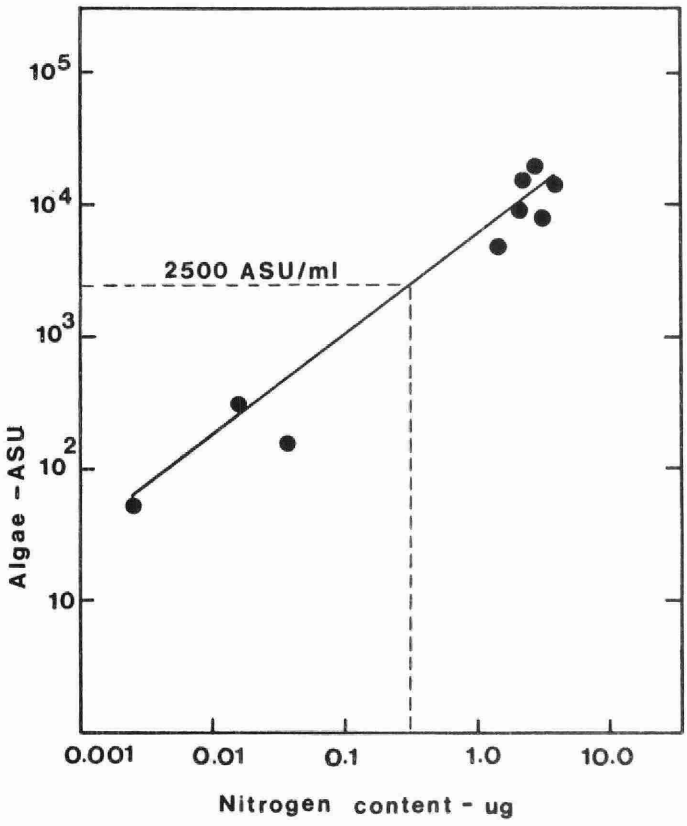

TABLE IV

The alkalinity of cultures after 21 days which received no additional inorganic carbon - initial alkalinity - 12 mg/l $CaCO_3$.

NO_3:N	Total Phosphorus		
	0.020	0.22	0.65
0.06	14		
9.60	14		
14.84	14	25	29
20.10		27	31

DISCUSSION AND CONCLUSION

The results of the above study (Table III) show that after an incubation period of 21 days, algal standing crops at least 100 times that of the control were obtained only when nitrogen and phosphorus were added in combination. Statistical analysis of data, obtained from replicated treatments, indicated no significant differences between duplicated samples or effects related to carbon availability, but did demonstrate a significant difference between responses associated with the lowest and two highest phosphorus levels, the latter also being found indistinguishable. The development with time of a culture receiving carbon, nitrogen and phosphorus (Figure 1), when compared to the growth of an unialgal culture (Fogg, 1964), indicates that cultures responding to fertilization were still displaying a positive exponential growth rate after 21 days, which suggests that further differences between treatments might have been obtained had the study been prolonged until all responses had attained a maximum.

The increase in alkalinity (mg/l $CaCO_3$) of those cultures receiving both nitrogen and phosphorus but no carbon, from 12 mg/l to at least 25 mg/l (Table IV) probably results

from the metabolic incorporation of nitrogen and phosphorus with the subsequent neutralization of the excess potassium ions by the formation of bicarbonate or carbonate ions. This induced alkalinity would appear to have masked the ability of carbon to act as a limiting nutrient for algal growth in low alkalinity surface waters (Birge and Juday, 1911; Christie, 1968), and future investigations to clarify this relationship must be carried out in such a manner as to minimize the development of this phenomenon.

The nitrogen content of the suspended solids when compared to the standing crops of algae displays a linear relationship having a correlation coefficient of 0,84. The nitrogen required in production of 2500 ASU/ml of phytoplankton, tentatively suggested as a maximum permissable algal concentration (Christie, 1968) is therefore 0.30 ug/ml, which is equivalent on a per litre basis to the value suggested by Sawyer (1947) for inorganic nitrogen at the time of the spring turnover in temperate lakes which will not support nuisance algal conditions.

No similar relationship could be developed from this data with respect to algal responses and total phosphorus; however, it is evident, when sufficient nitrogen is available,

that a total phosphorus level of 0.22 mg/l will support an abundance of algal growth whereas a concentration of 0.02 mg/l will not.

The results of this study tentatively suggest that the nutrients limiting the development of algae in this sample of lake water can be offset by the addition of both nitrogen and phosphorus. Investigations to substantiate the above relationship with respect to nitrogen and to develop a similar correlation with phosphorus and algae utilizing samples from relatively unproductive soft and hard surface waters as well as axenic cultures of nuisance algae, are now in progress. From this information, suitable criteria concerning levels of nitrogen and phosphorus in surface waters which will not support excessive productions of algae will be developed.

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